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Cardioselective sulfonylthiourea HMR 1098 blocks mitochondrial uncoupling induced by a K_{ATP} channel opener, P-1075, in beating rat hearts

Olga Jilkina[a], Bozena Kuzio[a], Gary J. Grover[b], Valery V. Kupriyanov[a,c,*]

[a] *Institute for Biodiagnostics, National Research Council, Winnipeg, Manitoba, Canada*

[b] *Cardiovascular and Metabolic Disease Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ, USA*

[c] *Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Manitoba, Canada*

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Abstract

We investigated effects of blockade of cardiac ATP-sensitive potassium channels (K_{ATP}) with a novel cardioselective sulfonylthiourea, HMR 1098, on metabolic uncoupling caused by a potent K_{ATP} opener, P-1075, in Langendorff-perfused rat hearts. We used (1) ^{87}Rb-NMR to detect activation–deactivation of sarcolemmal K_{ATP}, (2) ^{31}P-NMR to monitor high-energy phosphates, (3) oxygen uptake measurements to monitor cellular respiration, and (4) myocardial optical absorbance measurements at 603 nm to follow changes in cytochrome *c* oxidase redox state. Activation of sarcolemmal K_{ATP} by P-1075 (5 μM) and a mitochondrial uncoupler 2,4-dinitrophenol (DNP) (50 μM) stimulated Rb^+ efflux from the hearts by 130% and 60%, respectively. HMR 1098 (5 and 30 μM) blocked activation of sarcolemmal K_{ATP} in situ. HMR 1098 also prevented cardiac arrest and mitochondrial uncoupling induced by P-1075, such as (a) depletion of phosphocreatine and ATP by 40%, (b) two-fold decrease in venous oxygen, and (c) reduction of cytochrome *c* oxidase (demonstrated by an increase in 603 nm optical absorbance). The metabolic effects of P-1075 can be readily explained by activation of putative mitochondrial K_{ATP}. We concluded that blockade of mitochondrial uncoupling by HMR 1098 included an inhibiting effect of HMR 1098 on sarcolemmal and mitochondrial K_{ATP} in beating rat hearts.

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Keywords: ATP-sensitive potassium channel; Oxidative phosphorylation; Oxygen consumption; Cytochrome *c* oxidase; NMR; Optical spectroscopy

1. Introduction

ATP-sensitive potassium channels (K_{ATP}) are present in plasma membrane of a variety of cell types, where the channels serve as metabolic sensors, converting metabolic changes to changes in ionic fluxes and regulating sarcolemmal membrane excitability [1]. A second type of K_{ATP}, mitochondrial, is present in the inner mitochondrial membrane and is involved in the regulation of mitochondrial membrane potential ($\Delta\Psi$) [2]. Blockade of cardiac sarcolemmal K_{ATP} is known to protect against ischemia-induced fibrillation [3,4], while short-term activation of the mitochondrial K_{ATP} has been proposed to protect cardiac energetics and function during subsequent long-term ischemia [5,6]. Recently, Aventis Pharma, Frankfurt, Germany, synthesized a novel cardioselective K_{ATP} inhibitor, HMR 1098 (a free acid form, HMR 1883) [4]. This drug is a derivative of a well-known K_{ATP} inhibitor, glibenclamide that effectively blocks all types of K_{ATP} [2,3,6]. The non-aromatic substitutions in the sulfonylurea moiety greatly increased cardioselectivity of the drug and reduced the overall blocking potency, in comparison to glibenclamide [4,7]. In animal models, HMR 1098 exerted cardiodepressant effects, decreasing the incidences of ventricular tachycardia and ventricular fibrillation [4,8]. Based on measurements of flavoprotein fluorescence in isolated rabbit cardiomyocytes, it has been proposed that HMR 1098 lacks the ability to block mitochondrial K_{ATP} [9,10].

Abbreviations: DNP, 2,4-dinitrophenol; HR, heart rate; K_{ATP}, ATP-sensitive potassium channels; KHB, Krebs–Henseleit buffer; LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; PP, perfusion pressure

* Corresponding author. Institute for Biodiagnostics, National Research Council, 435 Ellice Avenue, Winnipeg, Manitoba, Canada R3B 1Y6. Tel.: +1-204-984-6620; fax: +1-204-984-7036.

E-mail address: Kupriyanov@ibd.nrc-cnrc.gc.ca (V.V. Kupriyanov).

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Our recent study, using a potent K_{ATP} opener, P-1075 (a derivative of pinacidil [11]), provided clear evidence that strong activation of K_{ATP} in intact beating rat hearts results in uncoupling of oxidative phosphorylation [12]. This effect was attributed to activation of mitochondrial K_{ATP}, because opening of sarcolemmal K_{ATP} per se has a cardioplegic effect, whereas opening of mitochondrial channels, depending on the degree of activation, may lead to depolarization of mitochondrial membrane, dissipation of $\Delta\Psi$, and a decrease in ATP production [12–15]. This makes P-1075 a very useful probe for testing the effects of other modulators of mitochondrial K_{ATP} in situ. In the present study, we intended to block sarcolemmal K_{ATP} in Langendorff-perfused rat hearts, using HMR 1098, and to investigate the effect of the blockade on P-1075-induced mitochondrial uncoupling. Unexpectedly, HMR 1098 blocked all mitochondrial effects of P-1075.

2. Materials and methods

The investigation conforms with the ''Guide to the Care and Use of Experimental Animals'' published by the Canadian Council on Animal Care (2nd edition, Ottawa, ON, 1993).

2.1. Reagents

Dimethylsulfoxide, 2,4-dinitrophenol (DNP), and RbCl were purchased from Sigma (St. Louis, MO, USA). P-1075 was synthesized in Bristol-Myers Squibb (BMS) Pharmaceutical Research Institute laboratories to be used for internal and collaborative use by BMS. HMR 1098 was a kind gift from Aventis Pharma. All other chemicals were of analytical grade. Stock solutions P-1075 were prepared in dimethylsulfoxide and further diluted in water. HMR 1098 was dissolved in water.

2.2. Heart perfusion

Male Sprague–Dawley rats (320–370 g) were anesthetized with pentobarbital solution (120 mg/kg). The hearts (1.4–1.7 g) were quickly removed and perfused retrogradely with phosphate-free KHB containing (in mM): 25 $NaHCO_3$, 118 NaCl, 4.7 KCl, 1.75 $CaCl_2$, 1.2 $MgSO_4$, 0.5 EDTA, and 11 glucose aerated with a mixture of 95% O_2 and 5% CO_2 to keep pO_2 at 500–600 mm Hg and pH at 7.4 at 36 °C. KHB-Rb had the same composition as KHB, except for K^+, which was substituted with Rb^+ by 50%.

Following the placement of a left ventricular apical drain, a latex balloon was inserted through the mitral valve into the left ventricular cavity and filled with H_2O (40–50 μl). The balloon was connected to a Statham P23Db pressure transducer and to a Digi-Med Model-210 heart performance analyzer (Micro-Med, Louisville, KY, USA) to monitor heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and perfusion pressure (PP). Pressure-rate product (PRP), calculated as developed pressure (LVSP minus LVEDP) multiplied by HR, was used as an index of mechanical work. The coronary flow rate was monitored using an ultrasonic blood flow meter (Transonic Systems Inc., Ithaca, NY, USA), and PP was measured continuously through the catheter connecting the aortic line and the second pressure transducer. Following a stabilization period, the hearts were perfused at a constant flow of 13–15 ml/min to provide the desired concentration of drugs during infusion.

2.3. Experimental protocols

In ^{31}P-NMR experiments, three initial 4-min ^{31}P spectra were acquired and used as a baseline. After that, P-1075 (5 μM) and HMR 1098 (5 or 30 μM) were infused for 20 min (five 4-min ^{31}P-NMR spectra), followed by a 16-min recovery period (four additional ^{31}P-NMR spectra). In ^{87}Rb-NMR experiments, initially all the hearts were loaded with Rb^+ by perfusing with KHB-Rb for 30 min. Rb^+ efflux was initiated by switching to a Rb^+-free KHB. Drug infusion started following a 4-min extracellular Rb^+-washout period and continued for an additional 20 min. In optical and oxygen consumption experiments, baseline values were measured for 12 min, followed by drug (P-1075, 5 μM, in the presence or absence of HMR 1098, 5 μM) infusion for 20 min, and 16-min recovery.

2.4. NMR spectroscopy

NMR experiments were performed using a Bruker AM-360 WB spectrometer equipped with a 20-mm Morris Instruments broadband probe placed in a wide bore vertical 8.4 T magnet. The ^{23}Na signal (95.25 MHz) from the heart and surrounding bath was used for adjusting homogeneity (shimming) of the magnetic field. ^{31}P-NMR spectra were acquired at 145.8 MHz using a 24-μs pulse length (60° flip angle), 2.0 s recycle time and 4 min resolution time. The sweep width was 10 kHz, memory size, 4 K data points, line broadening factor, 20 Hz. After baseline correction (spline), the heights and chemical shifts of the peaks of inorganic phosphate (P_i), phosphocreatine (PCr), and β- and γ-phosphate of ATP were measured using a peak-picking subroutine (Bruker). The heights of the peaks prior to the drug infusion were taken as 100%. A capillary containing 10 μl of 1 M solution of phenylphosphonic acid was used as a reference.

^{87}Rb-NMR spectra were acquired at 117.8 MHz every 2 min using a spectral sweep width of 18 kHz, a recycle time of 10 ms, and a pulse duration of 55 μs (90° flip angle). Memory size was 512 data points. Line broadening factor was 150 Hz. To minimize the signal from the extracardiac ^{87}Rb, a suction line was placed at the bottom of the NMR tube. A 10-μl capillary containing 1 M RbCl and 5 M KI was used as a reference.

2.5. Kinetics of Rb^+ efflux

A Rb^+ efflux rate constant (k) was calculated as a slope from a linear portion of a semilogarithmic plot (natural logarithm of Rb^+ peak intensity, a.u., vs. time, min) using a linear regression method in a Microsoft Excel program.

2.6. Oxygen consumption

An aliquot of the perfusate entering the heart was taken as an arterial sample. Venous effluent was collected from the cannulated right atrium. The oxygen content (pO_2, mm Hg) in the samples was measured at approximately 7-min intervals using Novastatprofile-Plus-9 (Nova Biomedical, MA, USA).

2.7. Myocardial optical absorbance

Optical absorbance was measured using InfraSpec Dermal System manufactured by the Institute for Biodiagnostics, National Research Council of Canada (Winnipeg, MB, Canada) equipped with a custom-made bifurcated fiber-optic cable. One end of the cable was connected to a source of white light (Fiber Optic Illuminator, model 77501, Oriel Instruments, Stratford, CT, USA), while a second end terminated at the detector. The individual fibers of the cable were combined into a common probe tip that was in a direct contact with the left ventricle. Non-gated spectra were acquired in the range 400–1000 nm every 30 s (60 scans). Spectral data were processed using Grams/32, Version 4.11 computer program (Galactic Industries Corp., USA).

2.8. Statistics

ANOVA (single factor) was used for data comparison. Differences were considered statistically significant when $P < 0.05$. Data are presented as means ± standard errors.

3. Results

3.1. Blockade by HMR 1098 of P-1075-induced activation of sarcolemmal K_{ATP}

Addition of P-1075 (5 μM) to the perfusate resulted in a cardiac arrest due to, perhaps, plasma membrane hyperpolarization induced by activation of sarcolemmal K_{ATP} in sinoatrial node. P-1075 increased the rate of Rb^+ efflux from Rb^+-loaded hearts more than two-fold: from $k = 0.040 \pm 0.001$ under basal conditions ($n = 3$) to 0.093 ± 0.001 ($n = 4$), min^{-1}. Simultaneous infusion of a cardioselective glibenclamide analog, HMR 1098 (5 μM) prevented cardiac arrest and abolished the Rb^+ efflux increase: $k = 0.046 \pm 0.002$, min^{-1} ($n = 3$) (Fig. 1). These data are consistent with activation of sarcolemmal K_{ATP} by P-1075 and their inhibition by HMR 1098 in cardiomyocytes in situ. HMR 1098 alone (5 μM) had no effect on the rate of Rb^+ efflux: $k = 0.045 \pm 0.002$, min^{-1} ($n = 3$), which indicates the closed state of sarcolemmal K_{ATP} under normal conditions. HMR 1098 also fully blocked the Rb^+ efflux increase due to an indirect activation of sarcolemmal K_{ATP} by a mitochondrial uncoupler, DNP (*via* a decrease in the [ATP]/[ADP] ratio). DNP (50 μM) increased k to 0.065 ± 0.002, min^{-1} ($n = 3$), while HMR 1098 (30 μM) returned the Rb^+ efflux to the basal level: $k = 0.042 \pm 0.006$, min^{-1} ($n = 3$).

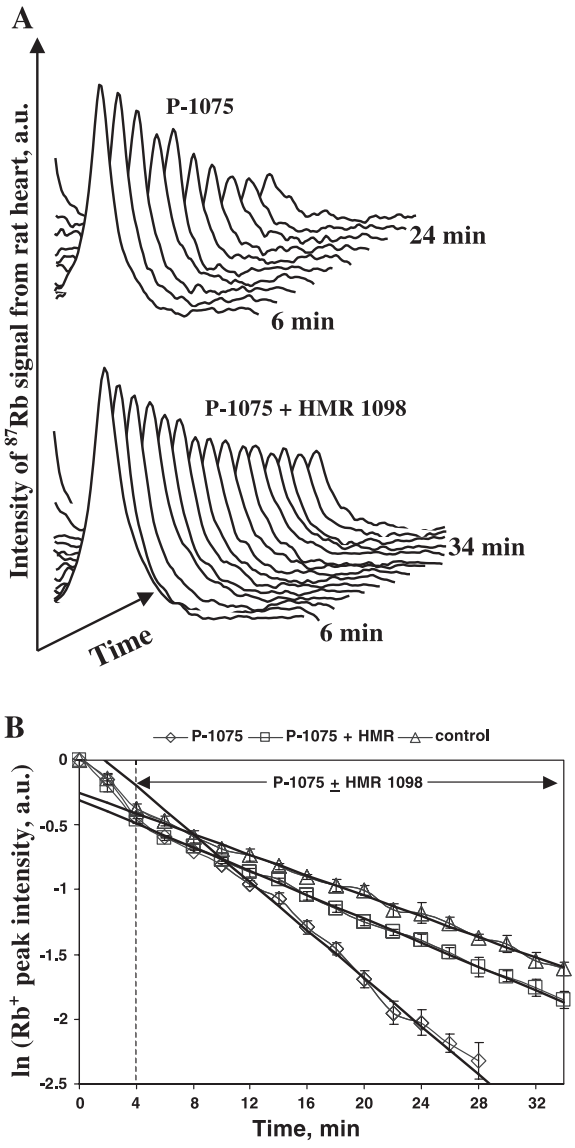

Fig. 1. Blockade by HMR 1098 of P-1075-induced activation of Rb^+ efflux in perfused rat hearts. (A) Representative ^{87}Rb-NMR spectra illustrating activated Rb^+ efflux from a perfused rat heart treated with P-1075 (5 μM) and reversal of the effect with HMR 1098 (5 μM). The hearts were loaded with Rb^+ as described under Materials and methods, and Rb^+-washout was initiated at time zero. Drug infusion started after the washout of extracardiac and extracellular Rb^+ (4 min of the Rb^+ washout). (B) Pooled data for ^{87}Rb NMR experiments. Data are presented as semilogarithmic plots and straight lines are linear fits for the data for control ($n = 3$), P-1075 (5 μM, $n = 4$)-, and P-1075 (5 μM) + HMR 1098 (5 μM, $n = 4$)-treated hearts.

3.2. Blockade by HMR 1098 of a P-1075-induced depletion of high-energy phosphates

The primary rationale for these experiments was to compare the proposed sarcolemmal specificity of HMR 1098 to its parent compound, glibenclamide. In addition to activation sarcolemmal K_{ATP}, in Langendorff-perfused beating rat hearts, P-1075, at a concentration of 5 μM, induced mitochondrial uncoupling [12]. Infusion of HMR 1098 prevented a 40% depletion of PCr and ATP and a two-fold increase in P_i (Fig. 2 and Table 1). Interaction of HMR 1098 with the SUR subunit of sarcolemmal K_{ATP} was reported to be markedly (7- to 100-fold) weaker in comparison to its parent compound, glibenclamide [16]. Glibenclamide (5 μM) was effective in blocking P-1075-induced mitochondrial effects in situ [12], thus initially HMR 1098 was used at a concentration of 30 μM. However, 30 μM HMR slightly, but significantly, decreased the level of PCr upon treatment (Table 1), probably due to an uncoupling effect of this compound at a high concentration, similar to that of glibenclamide [17]. Therefore, a lower (5 μM) concentration of HMR 1098 was tested as well. As summarized in Table 1, not only 30, but also 5 μM was a sufficient concentration of HMR 1098 in preventing depletion of high-energy phosphates. HMR 1098 alone (5 μM) had no significant effect on cardiac energetics (Table 1).

Table 1
Effects of P-1075 and HMR 1098 on high-energy phosphates

Group	Treatment (20 min)	
	PCr (%)	ATP (%)
Control ($n=5$)	93.2 ± 2.7	96.6 ± 3.9
P-1075, 5 μM ($n=7$)	60.3 ± 2.2*	59.7 ± 4.6*
HMR 1098, 5 μM ($n=3$)	100.9 ± 2.2	94.2 ± 2.7
HMR 1098, 30 μM ($n=3$)	76.5 ± 6.9*	93.2 ± 2.5
P-1075, 5 μM, +HMR 1098, 5 μM ($n=4$)	91.1 ± 5.9	94.1 ± 4.1
P-1075, 5 μM, +HMR 1098, 30 μM ($n=4$)	88.6 ± 6.8	91.7 ± 6.5

PCr and ATP levels before any interventions were set as 100%.
Mean ± S.E. are presented.
* Statistically significant vs. control.

3.3. Effects of HMR 1098 on oxygen consumption

In another series of experiments, oxygen consumption by the hearts was measured, under constant flow conditions. On P-1075 treatment, oxygen concentration in venous effluent reversibly decreased two-fold (Fig. 3), indicating that mitochondrial uncoupling rather then inhibition of electron transport was the cause of ATP depletion [12]. Stimulation of cellular respiration persisted until the removal of P-1075 (not shown). Consistent with the results of ^{31}P-NMR experiments, HMR 1098 eliminated the P-1075-induced increase in heart's oxygen consumption (Fig. 3).

3.4. Effect of HMR 1098 on a P-1075-induced reduction of cytochrome c oxidase

Simultaneous with the oxygen consumption measurements, myocardial absorbance data were acquired. Absorbance at 603 nm was measured as an index of cytochrome *c* oxidase (cyt aa_3) redox state [18]. On P-1075 treatment, cytochrome *c* oxidase became reduced: a reversible transitory increase was observed in the absorbance at 603 nm, but not at other wavelengths (Fig. 4A). On average, a change in

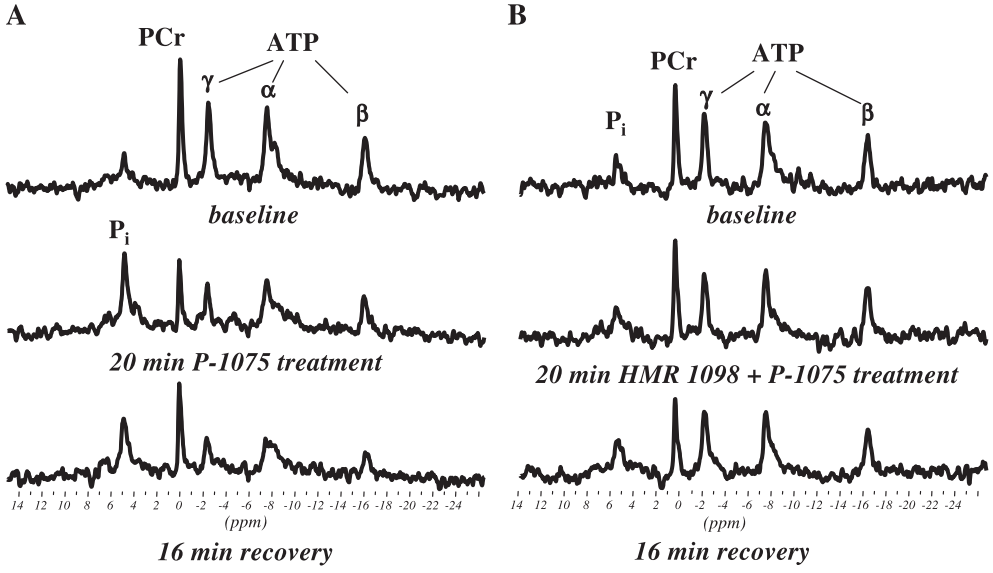

Fig. 2. Blockade by HMR 1098 of P-1075-induced changes in high-energy phosphates in perfused rat hearts. Representative ^{31}P-NMR spectra of the hearts treated with P-1075 (5 μM) in the absence (panel A) or presence of HMR 1098 (5 μM, panel B). The spectra are a sum of 116 acquisitions collected over 4-min periods before (baseline), 20-min after drug infusion, and 16-min after recovery. Chemical shifts are given with reference to that of the phosphocreatine (PCr) peak, which is set at zero. P_i, inorganic phosphate.

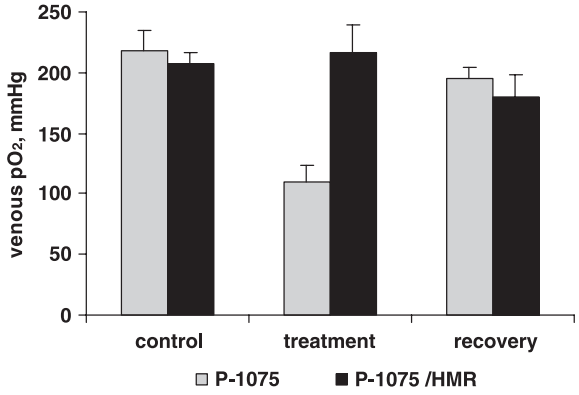

Fig. 3. The effects P-1075 (5 μM, $n=3$) on venous oxygen pressure in the absence and in the presence of HMR 1098 (5 μM, $n=3$), in perfused rat hearts. Venous effluent was collected from the cannulated right atrium and oxygen content measured before (control), after 20-min drug infusion (treatment), and after 16-min recovery.

absorbance at 603 nm, induced by P-1075, was 29.6 ± 4.8% ($n=3$), in comparison to that induced by global no-flow ischemia (Fig. 4A). The effect of P-1075 was also fully blocked by HMR 1098 (Fig. 4B).

In comparison, 50 μM DNP induced a similar 603 nm absorbance increase (Fig. 4C). However, the kinetics was different: while the change in 603 nm absorbance induced by P-1075 was transitory and cytochrome *c* oxidase redox state returned to the basal level before the P-1075 washout, on DNP application (20 min), cytochrome *c* oxidase remained reduced until the removal of DNP (Fig. 4C). DNP-induced 603 nm absorbance change was 37.9 ± 5.8% ($n=3$) of that induced by ischemia.

3.5. Mechanical function of Langendorff-perfused rat hearts on drug treatment

There were no significant differences in the baseline parameters of hearts in all groups: typically, HR was approximately 275 beats/min; LVEDP 7, LVSP 110, and PP 70 mm Hg. Addition of 5 μM P-1075 resulted in cardiac arrest (after approximately 9 min). During 20-min treatment, P-1075 caused a marked increase in LVEDP (Fig. 5A) and a decrease in LVSP (Fig. 5B); systolic and diastolic pressures became equal following cardiac arrest. The effects on LVSP and LVEDP were significantly attenuated by the infusion of

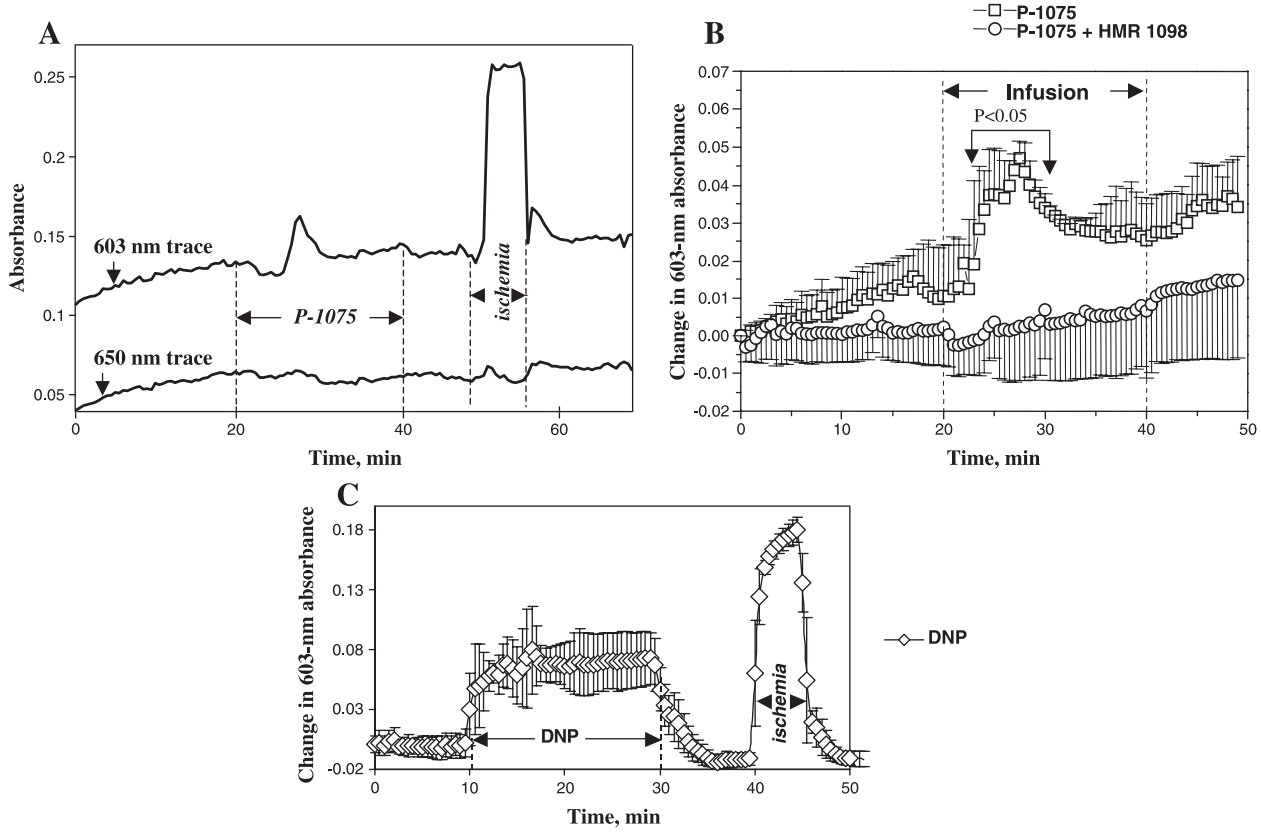

Fig. 4. Effect of P-1075 (in the absence or presence of HMR 1098) on cytochrome *c* oxidase redox state. (A) Time course of the absorbance at 603 (top trace) and 650 nm (bottom trace) from a representative heart treated with P-1075. P-1075 (5 μM) was infused between 20 and 40 min of the experiment. The heart was subjected to global no-flow ischemia between 50 and 55 min of the protocol to calibrate the changes caused by P-1075. Absorbance at 603 nm was indicative of the redox state of cytochrome *c* oxidase. Absorbance at 650 nm was chosen to demonstrate non-specific changes in the heart optical properties. (B) Pooled data for the hearts treated with P-1075 (5 μM, $n=3$) and P-1075 (5 μM) + HMR 1098 (5 μM, $n=3$). $P<0.05$ is indicated for data in "P-1075-only" group vs. "P-1075 + HMR 1098" group. (C) Pooled data for the hearts treated with DNP (50 μM, $n=3$) and subjected to global no-flow ischemia to calibrate the changes caused by DNP.

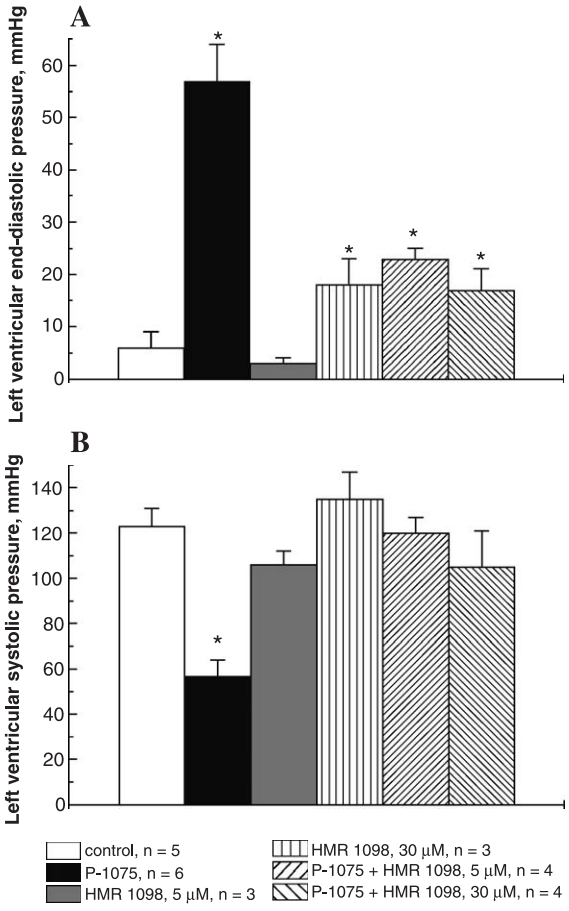

Fig. 5. Summarized data on left ventricular end-diastolic and systolic pressures of hearts treated with P-1075 (5 μM) and HMR 1098. The hearts were treated for 20 min with the HMR 1098 at indicated concentrations and the values correspond to 20 min of drug treatment.

HMR (5 and 30 μM) and the hearts did not stop beating (Fig. 5). HMR 1098 alone (30 μM) slightly, but statistically significantly, increased LVEDP; however, 5 μM had no effect (Fig. 5A).

4. Discussion

To investigate cardiac effects of HMR 1098 in situ, we used a combination of non-invasive methods, namely: (1) ^{87}Rb-NMR spectroscopy to detect activation–deactivation of sarcolemmal K_{ATP}, (2) ^{31}P-NMR spectroscopy to monitor high-energy phosphates, (3) oxygen uptake measurements to monitor cellular respiration, and (4) myocardial optical absorbance at 603 nm to follow changes in cytochrome *c* oxidase redox state [12,18,19]. The results of all these experiments clearly demonstrated that HMR 1098 blocks not only sarcolemmal, but also mitochondrial effects induced by a K_{ATP} opener, P-1075, in the hearts.

In addition to already established effects of P-1075 on high-energy phosphates, oxygen uptake, and contractile parameters [12], this drug also caused transient reduction of cytochrome *c* oxidase. This is consistent with mitochondrial uncoupling, which facilitates faster movement of redox equivalents to cytochrome *c* oxidase causing its reduction. Return of the redox state to the baseline prior to P-1075 washout (Fig. 4) probably reflects changes in the kinetic properties of cytochrome *c* oxidase and Krebs cycle in a response to the [ATP]/[ADP] decrease, [P_i] and [H^+] increases, mitochondrial swelling [20], as well as changes in some unknown factors, triggered by cardiac arrest. For example, inhibition of Krebs cycle and/or dehydrogenases would reoxidize cytochrome *c* oxidase.

The observation that P-1075 binds with high affinity to myocardial membrane preparations (containing sarcolemmal and mitochondrial membranes) in MgATP-dependent and glibenclamide-sensitive manner [21], provides evidence for the interaction of P-1075 with native cardiac K_{ATP}. There is no doubt that P-1075 interacts with native sarcolemmal K_{ATP}. P-1075 is the only K_{ATP} opener, whose significant pharmacological binding to recombinant sarcolemmal K_{ATP} was demonstrated [22,23]. However, interaction of P-1075 with native mitochondrial K_{ATP} has not been demonstrated directly. Moreover, up to date, the molecular identity of mitochondrial K_{ATP} has not been established. The existence of glibenclamide-sensitive mitochondrial K_{ATP} was suggested based on (a) patch–clamp experiments on fused mitoplasts [2], (b) measurements of K^+ fluxes through mitochondrial channel fraction reconstituted in lipid membranes [24], (c) mitochondrial swelling [20] and (d) pharmacological studies involving presumably specific mitochondrial K_{ATP} modulators, diazoxide and 5-hydroxydecanoate (5-HD) [5,6]. In addition, uncoupling induced by several K_{ATP} openers in isolated mitochondria [13,15] and quiescent rabbit cardiomyocytes was attributed to the activation of mitochondria K_{ATP} [14]. Recently, however, non-channel metabolic effects of diazoxide and 5-HD were demonstrated [25,26]. Moreover, the validity of the models allowing indirect measurements of mitochondrial K_{ATP} activity was questioned [27–30], thus, further signifying that until these channels are identified at the molecular level, interpretation of the data leaves room for discussion. Nevertheless, glibenclamide binding to mitochondria provides another indirect evidence for the existence of mitochondrial K_{ATP} [31], since highly specific interaction of glibenclamide with the sulfonylurea receptor of K_{ATP} is well established [3,6].

Unlike DNP-induced uncoupling of oxidative phosphorylation, uncoupling by P-1075 was completely inhibited by glibenclamide [12] and HMR 1098 (this work), which clearly indicated involvement of K_{ATP}, either mitochondrial or sarcolemmal. One cannot completely discard the possibility that activation of sarcolemmal K_{ATP} could contribute towards the metabolic effects of P-1075 via generation of an "uncoupling factor." The missing link in this hypothesis is the nature and mechanism of generation of the factor. Elevated cytoplasmic Ca^{++} (entered through sarcolemma or released from sarcoplasmic reticulum) and/or free fatty acids (due to activation of lipolysis of endogenous lipids) can impair

ATP production. However, cell membrane hyperpolarization that likely resulted from activation of sarcolemmal K_{ATP} by P-1075, would rather decrease Ca^{++} entry [32], and there is no data to suggest K_{ATP} openers-dependent Ca^{++} release from sarcoplasmic reticulum, or lipolysis. For these reasons, activation of putative mitochondrial K_{ATP} by P-1075 and depolarization of mitochondrial membrane seems to be a more plausible hypothesis. Blockade of the P-1075 metabolic effects by the sulfonylureas also demonstrated that at the concentration used in this study, any possible non-K_{ATP} effects of P-1075 on cardiac metabolism were negligible.

Sarcolemmal specificity of HMR 1098 was initially proposed, based on the measurements of mitochondrial flavoprotein fluorescence in isolated quiescent rabbit cardiomyocytes [9]. It was demonstrated that HMR 1098 did not affect diazoxide- and pinacidil-induced flavoprotein oxidation in these cells [9,10]. However, it has to be noted that the same model did not allow measurements of a well-established effect of glibenclamide [2] on mitochondrial K_{ATP} [9,10] and, thus, may not detect the effect of a related drug, HMR 1098. In addition, ability of HMR 1098 to block a K_{ATP} opener-induced mitochondrial uncoupling may be different in quiescent rabbit cardiomyocytes and beating rat hearts due to a different energetic status ([ATP]/[ADP] ratio), Ca^{++} levels, and/or other presently unknown factors. Indeed, state-dependent effects of glibenclamide on mitochondrial K_{ATP} were reported [33]. Finally, flavoprotein fluorescence [9,10] and high-energy phosphates (Ref. [12] and this work) may respond differently to treatment with K_{ATP} effectors (e.g., glibenclamide and P-1075). It was reported also that in lipid bilayers containing phosphatidylethanolamine and phosphatidylserine, reconstituted mitochondrial K_{ATP} could not be blocked with HMR 1098 [34]. However, this model did not include cardiolipin, which is the only phospholipid localized exclusively in mammalian mitochondria and involved in the modulation of a number of mitochondrial functions [35].

Additional evidence to support sarcolemmal specificity of HMR 1098 comes from the studies of ischemic and pharmacological preconditioning, the phenomenon presumably involving mitochondrial K_{ATP} [5,6]. However, preconditioning experiments do not provide direct measurement of mitochondrial K_{ATP} activity. In addition, these studies are ambiguous, as contradictory results have been reported. Several researchers observed no effect of HMR 1098 on different types of preconditioning [36–42]. However, others demonstrated that HMR 1098, attenuated or blocked protection by ischemic, K_{ATP}-opener- or isoflurane-induced myocardial preconditioning in rabbits [43–45], desflurane-induced cardioprotection in dogs [46], and delayed opioid-induced preconditioning in rats [47], raising doubts that HMR 1098 blocks only sarcolemmal K_{ATP}. Alternatively, these reports leave room for the interpretation of the results, as to whether not only mitochondrial, but also sarcolemmal K_{ATP} are involved in a variety of types of preconditioning. Interestingly, Suzuki et al. [48] demonstrated the absence of ischemic preconditioning in Kir6.2$^{-/-}$ transgenic mice lacking sarcolemmal, but not mitochondrial K_{ATP}, thus, further signifying the role of sarcolemmal membrane properties in cardioprotection. This group proposed the phenomenon to be typical of mice, whose heart rate is high (~ 600 beats/min), however, sarcolemmal K_{ATP} may play a crucial role in cardioprotection of other species.

In summary it was demonstrated that HMR 1098 blocks mitochondrial uncoupling by P-1075, assessed by depletion of PCr and ATP, decreased venous oxygen, and reduction of cytochrome *c* oxidase in Langendorff-perfused rat hearts.

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